CONJUGATION PROCESS OF BACTERIAL POLYSACCHARIDES TO CARRIER PROTEINS

This application is filed as a United States Continuation Application which claims priority to U.S. application Ser. No. 13/581,824 filed Aug. 30, 2012, now U.S. Pat. No. 8,753,645 issued Jun. 17, 2014, which was filed pursuant to 35 U.S.C. §371 as a United States National Phase Application of International Patent Application Serial No. PCT/EP2011/053400 filed Mar. 7, 2011, which claims priority to United Kingdom Application No. 1003922.0 filed Mar. 9, 2010; the entire contents of each of the foregoing applications are hereby incorporated by reference.

The present invention relates to a process for conjugation. In particular, it relates to the conjugation of saccharides and proteins using reductive amination.

BACKGROUND

Bacterial capsular polysaccharides have been widely used in immunology for many years for the prevention of bacterial disease. A problem with such a use, however, is the T-independent nature of the immune response. These antigens are thus poorly immunogenic in young children. This problem has been overcome through conjugating the polysaccharide antigens to a carrier protein (a source of T-helper epitopes) which may then be used to elicit a T-dependent immune response, even in the first year of life.

Various conjugation techniques are known in the art. Conjugates can be prepared by direct reductive amination methods as described in, US200710184072 (Hausdorff) U.S. Pat. No. 4,365,170 (Jennings) and U.S. Pat. No. 4,673,574 (Anderson). Other methods are described in EP-0-161-188, EP-208375 and EP-0-477508. The conjugation method may alternatively rely on activation of hydroxyl groups of the saccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. Such conjugates are described in PCT published application WO 93/15760 Uniformed Services University and WO 95/08348 and WO 96/29094. See also Chu C. et al Infect. Immunity, 1983 245 256.

Reductive amination involves two steps, (1) oxidation of the antigen, (2) reduction of the antigen and a carrier protein to form a conjugate. The oxidation step may involve reaction with periodate, however oxidation by periodate may lead to size reduction (WO94/05325).

SUMMARY OF INVENTION

The inventors have surprisingly found that using lower concentrations of periodate in the presence of low phosphate may lead to retention of size and/or the retention of epitopes.

In a first aspect of the invention there is provided a process for conjugating a bacterial saccharide(s) comprising the steps of

a) reacting the bacterial saccharide with 0.001-0.7, 0.005-0.5, 0.01-0.5, 0.1-1.2, 0.1-0.5, 0.1-0.2, 0.5-0.8, 0.1-0.8, 0.3-1.0 or 0.4-0.9 molar equivalents of periodate to form an activated bacterial saccharide;

b) mixing the activated bacterial saccharide with a carrier protein;

c) reacting the activated bacterial saccharide and the carrier protein with a reducing agent to form a conjugate;

or

a) reacting the bacterial saccharide with 0.001-0.7, 0.005-0.5, 0.01-0.5, 0.1-1.2, 0.1-0.5, 0.1-0.2, 0.5-0.8, 0.1-0.8, 0.3-1.0 or 0.4-0.9 molar equivalents of periodate to form an activated bacterial saccharide;

b) mixing the activated bacterial saccharide with a linker;

c') reacting the activated bacterial saccharide with the linker using a reducing agent to form a bacterial saccharide-linker;

d) reacting the bacterial saccharide-linker with a carrier protein to form a conjugate;

wherein step a) occurs in a buffer which does not contain an amine group, and the buffer has a concentration between 1-100 mM.

In a second aspect of the invention there is provided a conjugate obtainable by the process of the invention.

In a third aspect of the invention there is provided a conjugate obtained by the process of the invention.

In a fourth aspect of the invention there is provided an immunogenic composition comprising the conjugate of the invention and a pharmaceutically acceptable excipient.

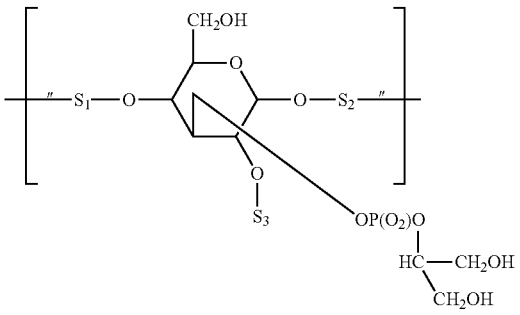
In a fifth aspect of the invention there is provided a vaccine comprising the immunogenic composition of the invention.

In a sixth aspect of the invention there is provided a use of the immunogenic composition of the invention or the vaccine of the invention in the prevention or treatment of bacterial disease

In a seventh aspect of the invention there is provided a use of the immunogenic composition of the invention or the vaccine of the invention in the preparation of a medicament for the prevention or treatment of bacterial disease.

In a eighth aspect of the invention there is provided a method of preventing or treating bacterial infection comprising administration of the immunogenic composition of the invention or the vaccine of the invention to a patient.

In an ninth aspect of the invention there is provided an activated bacterial saccharide, wherein the activated bacterial saccharide comprises a repeat unit of formula (I):



wherein the activated bacterial saccharide comprises n repeat units and n is between 2 and 2400, between 500 and 2000, between 750 and 1500, between 1000 and 2000 or between 1500 and 2300.

wherein at least 0.001%, 0.01%, 0.1%, 0.5%, 1%, 2%, 5%, 10% or 30% but less than 0.001%, 0.01%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 30% or 50% of S1 is